

Specific inhibition of IgE antibody production by an antisense oligodeoxynucleotide oligomer (OligostickTM)

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SUMMARY

We have investigated the ability of an antisense oligonucleotide (ASE-1) to specifically inhibit IgE synthesis by a human myeloma cell line, U266. ASE-1 inhibited IgE production in a concentration-dependent manner, as assessed by isotype-specific ELISA measurement of immunoglobulin in myeloma cell supernatants. Inhibition of IgE production was specific and not due to cytotoxicity since IgG1 and IgM production by human myeloma cell lines ARH-77 and RPMI-1788 respectively, was not significantly affected by up to 20 μ M ASE-1 whereas IgE production was inhibited by approximately 70% at this concentration. These results indicate that antisense oligonucleotides represent a potential therapeutic approach to the treatment of IgE-mediated allergic diseases.

In atopic individuals, IgE antibodies produced against innocuous environmental antigens (allergens) play a central role in the aetiology of human allergic disorders.¹ Consequently, numerous strategies have been examined for their ability to specifically inhibit IgE antibody production, e.g. modified allergens,² IgE-derived peptides,³ anti-idiotypic antibodies,⁴ with varying degrees of success. Synthetic antisense oligonucleotide analogues (oligos) complementary to specific cellular, viral, parasite or oncogene mRNA sequences have been shown to selectively inhibit protein synthesis in *in vitro* culture systems and consequently antisense oligos may have considerable potential as therapeutic agents.⁵ We have examined the ability of an oligo, complementary to the initial 5' sequence of the C_H1 domain of the human IgE heavy chain gene, to regulate IgE antibody production by U266 myeloma cells. A 12-mer synthetic OligostickTM oligo (ASE-1; with an acridine-derivative intercalating agent linked to its 3' end) was selected for these experiments for several reasons: the small size facilitates entry into cells, and the recognition selectivity and affinity for the target sequence is enhanced by the intercalating agent, which also protects the oligo against exonuclease attack.^{5,6}

The human myeloma cell lines U266 (kindly provided by Dr N. K. Nilsson), ARH-77 and RPMI-1788 (ATCC, Rockville, MD) produce IgE λ , IgG κ and IgM λ antibodies, respectively. The 2-methoxy, 6-chloro, 9-amino acridine-conjugated OligostickTM 12-mer oligo (Appligene, Illkirch, France) referred to as ASE-1, had the following sequence: 3'-acr-CGGAGGT-GTGTC-5', which is complementary to the initial 5' sequence of the C_H1 domain of the human IgE heavy chain gene.⁷ The myeloma cell lines were cultured (2 \times 10⁴/200 μ l/well) with various concentrations of ASE-1 for 3 days in RPMI-1640

medium containing 2 mM glutamine, 50 U/ml penicillin, 50 μ g/ml streptomycin, 20 μ M 2-mercaptoethanol and 10% foetal calf serum (FCS) (Gibco, Paisley, U.K.). The amount of antibody in culture supernatants was quantitated by ELISA as follows: half-area EIA plates (Costar, Cambridge, MA) were coated with goat anti-human IgE, IgG or IgM antibodies (Nordic, Maidenhead, U.K.; 10 μ g/ml, 50 μ l/well) in pH 9.6 carbonate buffer overnight at room temperature. After washing with phosphate-buffered saline (PBS) + Tween 20, dilutions of cell-free supernatants and immunoglobulin standards [IgG and IgM from Sigma Chemical Co. (Poole, U.K.), and the WHO IgE serum standard] were used to generate immunoglobulin standard concentration curves for quantitation of Ig levels in myeloma cell supernatants were added and incubated for 3 hr. After washing, isotype-specific peroxidase-conjugated goat anti-human antibodies (Nordic) were added for 3 hr. Following washing, 50 μ l of 2.2 mM *o*-phenyldiamine (Sigma) containing 0.012% H₂O₂ was added to the wells for 30 min before the reaction was terminated by adding 25 μ l of 3 M H₂SO₄ when colour development was measured on a Dynatech Multiplate Reader at 492 nm (Billinghurst, U.K.). Antibody concentrations in culture supernatants were computed from immunoglobulin standard curves that were included on each ELISA plate. The amount of preformed antibody present in 1 \times 10⁵ myeloma cells, released into 1 ml of complete medium + FCS by five freeze-thaw cycles, was also determined in each experiment and these values (see legends of Fig. 1, Table 1) were subtracted from the data shown.

As shown in Fig. 1, ASE-1 inhibited IgE production by U266 cells in concentration-dependent fashion, IgE levels being significantly reduced by 10 μ M (30%) and 20 μ M (70%) ASE-1. In contrast to its inhibitory effect on IgE production, ASE-1 had no significant effect on IgG1 or IgM production at 20 μ M, the highest concentration tested in these experiments, showing the

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Table 1. ASE-1 does not affect human myeloma cell proliferation or viability

Cell line	Supernatant Ig (ng/ml)		Cell number/well ($\times 10^{-4}$)		Cell viability (%)	
	+0	+ASE-1	+0	+ASE-1	+0	+ASE-1
U266 (IgE)	1237 \pm 106	346 \pm 23 (72%)	5.2 \pm 0.4	5.6 \pm 0.5	88 \pm 2	85 \pm 3
ARH-77 (IgG1)	850 \pm 71	772 \pm 64 (9%)	4.4 \pm 0.5	4.0 \pm 0.4	87 \pm 3	84 \pm 2
RPMI-1788 (IgM)	1730 \pm 75	1649 \pm 154 (5%)	6.0 \pm 0.4	6.2 \pm 0.6	90 \pm 6	93 \pm 2

The cells were grown in the presence or absence of 20 μ M ASE-1 for 3 days. Supernatant immunoglobulin (Ig) levels were quantitated by isotype-specific ELISA. Cell numbers and viability were determined by trypan blue exclusion using light microscopy. The results are expressed as mean \pm 1 SD of triplicate cultures and preformed cellular antibody levels (in ng/ml: U266 = 62 \pm 10, ARH-77 = 21 \pm 3; RPMI-1788 = 37 \pm 10) have been subtracted. The figures in brackets show the per cent inhibition of Ig production by ASE-1 compared to control.

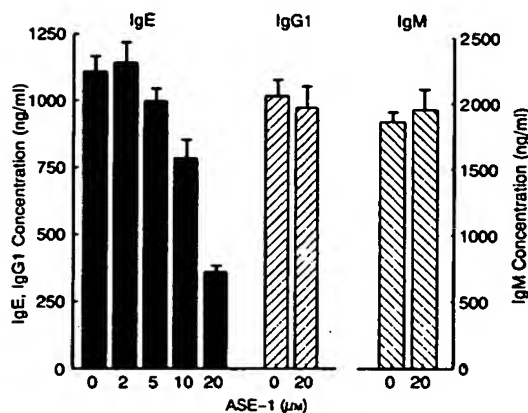


Figure 1. Human myeloma cells U266 (IgE), ARH-77 (IgG1) and RPMI-1788 (IgM) were cultured for 3 days in the presence or absence of an antisense oligonucleotide (ASE-1). Immunoglobulin levels were quantitated by isotype-specific ELISA. The results are expressed as mean \pm 1 SD of triplicate cultures and preformed cellular antibody levels (in ng/ml: U266 = 50 \pm 13, ARH-77 = 28 \pm 6, RPMI-1788 = 32 \pm 11) have been subtracted.

specificity of the oligo for inhibition of IgE synthesis. In the experiment shown in Table 1, ASE-1 (20 μ M) inhibited IgE production by U266 cells by 72%, but had no significant effect on U266 cell numbers or viability indicating that the inhibitory effect on IgE production was not due to a toxic effect on the cells. ASE-1 had no significant effect on IgG1 or IgM antibody production and did not significantly affect the proliferation or viability of ARH-77 or RPMI-1788 cells (Table 1).

The inhibition of IgE production by ASE-1 was remarkably selective, particularly in view of the close homology between the initial 5' sequences of the C_H1 domains of human IgE and IgG1, which differ at only two of 12 nucleotides (IgE 5'-GCCTCCACACAG-3'; IgG1 5'-GCCTCCACCAAG-3'). The lack of effect of ASE-1 on IgM production was not unexpected since the comparable sequences in IgE and IgM differ at 10 of 12 nucleotides (IgM 5'-TCAGGGAGTGCA-3'). Inhibition of IgE production was not due to a toxic effect of ASE-1 on cells (Table 1), nor can it be attributed to inhibition of λ -light chain synthesis, since RPMI-1788 antibody production (IgM λ) was unaffected by ASE-1. In the experiments reported here, IgE

production was inhibited when measured at Day 3 of culture, indicating that ASE-1 was not rapidly degraded or inactivated in the cultures or upon entry into cells. In preliminary experiments, we have found that IgE production was also selectively inhibited by 66% at Day 1 and 72% at 2 days of culture by 20 μ M ASE-1, suggesting that ASE-1 enters the cells and exerts its inhibitory effect(s) quite rapidly.

Antisense oligos have been shown to be effective in selectively inhibiting protein production in a wide variety of *in vitro* systems,^{5,10} but the *in vivo* potential of these compounds remains to be determined.^{10,11} However, with continued advances in the chemistry of deoxynucleotide analogues it is possible that the application of antisense oligos to the treatment of various clinical disorders, including IgE-mediated allergic conditions, will be realised in the future.¹¹

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